Microscope Cell Culture Incubator

BME 400

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Client: Dr. John Puccinelli, PhD **Advisor:** Dr. Paul Campagnola, PhD

Abstract

Live cell imaging allows researchers to precisely monitor temporal changes in cell morphology and behavior. To image live cells for extended periods of time, the temperature, pH, and concentration of their media must be maintained at optimal levels. Cell culture incubators typically maintain these conditions, but such devices do not fit on microscope stages. Therefore, our client, Dr. Puccinelli, wants the team to create an incubator modified to fit on a Nikon TI-U microscope stage without blocking the path of light from the microscope. This paper describes the team's process in developing a preliminary design for the on-stage incubator. The team plans to fabricate, test, and modify the described device.

Introduction

- Microscope cell culture incubator is important for cellular imaging over extended periods of time
- Eliminates need to remove cells from incubator to microscope and vice versa and better preserves cells
- Existing devices cost upwards of $10,000

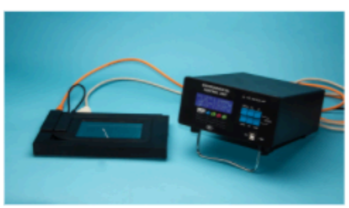

Figure 1: The Ibidi Stagetop Incubation System costs $13,990

- Importance of incubator parameters:
  - Temperature (37°C)
    - Maintains viability and healthy metabolic rate
  - pH (7.2-7.4)
    - Maintains cell viability and function
    - Corresponds to 5±1% CO_2 concentration
  - Relative Humidity (over 95%)
    - Prevents media evaporation
      - Maintain concentration of salts and analytes in media
    - Primarily dependent on number of times incubator is opened

Design Criteria

Client Requirements:

- Maintain 37°C, 100% Humidity, and 5% CO2 concentration
- Does not impede the optical path
- Allow cell plates with a maximum size of 130mm x 90mm x 20mm
- Uniform heating throughout the incubator
- Easy readout of conditions
- Ability to change out cell cultures
- Ability to be sterilized
- Combined budget: $100

Final Design

Design Matrices:

Table 1 - Box Design Matrix

		Design Element		
Categories	Weight	Plastic with Insulation	Metal with Insulation	Acrylic Glass
Heat Insulation	30	4/5 (24)	5/5 (30)	3/5 (18)
Cost	25	5/5 (25)	3/5 (15)	4/5 (20)
Sterilizable	20	5/5 (20)	4/5 (16)	1/5 (4)
Ease of Fabrication	15	5/5 (15)	5/5 (15)	5/5 (15)
Durability	10	3/5 (6)	2/5 (4)	4/5 (8)
Total	100	90	80	65

Table 2 - Plastic Design Matrix

		Design Element		
Categories	Weight	Acrylonitrile butadiene styrene (ABS)	High Density Polyethylene (HDPE)	Polypropylene (PP)
Cost	25	2/5 (10)	4/5 (20)	5/5 (25)
Heat Insulation	25	4/5 (25)	5/5 (25)	3/5 (12)
Sterilizable	20	5/5 (20)	2/5 (8)	5/5 (20)
Ease of Fabrication	15	4/5 (12)	5/5 (15)	5/5 (15)
Durability	15	5/5 (15)	3/5 (9)	4/5 (12)
Total	100	82	77	84

The inner casing of the incubator will be plastic because it is cheap to manufacture and has the ability to withstand high temperatures for long periods of time. The outer casing will be styrofoam for further insulation. Polypropylene will be used as the plastic as it is relatively durable, cheap, and easy to sterilize in an autoclave.

Materials:

- 5 Polypropylene sheets 12in x 12in x 1in
- Screws
- 2 pieces acrylic glass
- MH-Z16 CO2 sensor
- DHT-22 Temperature/Humidity sensor
- (¼") Gas Solenoid Valve
- Grove Water Atomizer
- Heating Element
- Arduino

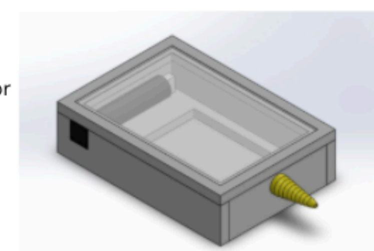

Figure 2: SolidWorks rendering of Final Design

Fabrication:

- Will be made next semester in the Team Lab

Testing

Tests:

- Simulate temperature distribution in cell culture plate using COMSOL
- Collect data on CO_2 Pressure, Temperature, and Humidity of microscope incubator over duration of trial
- Run Statistical T Tests and ANOVA tests to compare means and variance, respectively

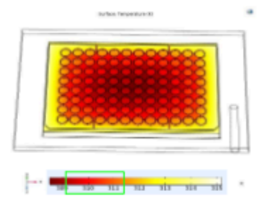

Figure 3: Simulated heat distribution using water immersion heating element

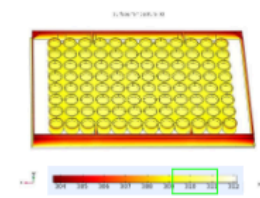

Figure 4: Simulated heat distribution using transparent heating element under plate

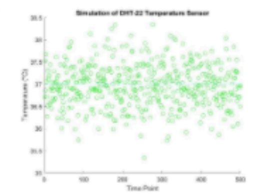

Figure 5: Expected temperature measured by the temperature - humidity sensor over time

Coding and Circuitry

Arduino Code:

- Temperature control
- Relative humidity
  - Used to reduce vapor pressure of liquids in culture
- CO_2 sensor read in and control

Circuit and Other Electronics:

- DHT temperature and humidity sensor
- MH-Z16 CO_2 Sensor
- Water Atomizer to create humidity
- Heating Element

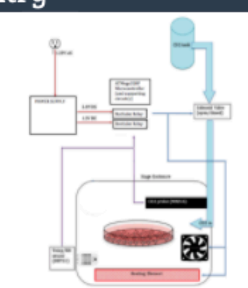

Figure 6: System Control Diagram

Future Work

- Fabrication of incubator box in TEAM lab
- Program coding for microcontroller
- Construct circuit for code and incubator box
- Measure accuracy of sensors and incubator variables
- Activate microscope incubator for up to 7 days
- Run statistical analysis on raw data

TEAMLab Technical Education and Manufacturing

Figure 7: UW Madison Team Lab

Acknowledgements

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UW-Madison BME Design

Sources

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